REMARKS

I. INTRODUCTION

Claims 31 and 36 have been amended. Claims 1-30 and 34-35 were previously cancelled. Claims 41-67 were withdrawn. Thus, claims 31-33 and 36-67 are pending in the present application. No new matter has been added. In view of the above amendments and the following remarks, it is respectfully submitted that claims 31-33 and 36-40 are in condition for allowance.

II. THE RESTRICTION REQUIREMENT SHOULD BE WITHDRAWN

The Examiner again responds to the arguments presented relating to the Restriction Requirement. (See 6/23/10 Office Action, p. 14). Although the Examiner again does not explicitly re-present the restriction requirement, Applicants maintain the position that the claims are of a single general inventive concept under PCT Rule 13.1. Applicants again respectfully maintain all of the previously presented arguments regarding withdrawal of the restriction requirement.

The Examiner maintains that the common technical feature among the different purported groups is the culture media and that the intended use of the composition for the expansion of stem cells is not a feature of all the groups. Regarding the intended use, it is respectfully maintained that the intended use is not necessarily the common technical feature among the groups. The Examiner seems to agree that the culture media is the common feature. The culture media itself is what provides the improved results of using the culture media of the present invention for expanding autologous human stem cells to be realized. The Examiner further stated that in view of Xia, the claimed composition of the present invention is merely an obvious variant and differs only in the concentration of the heparin component. As discussed with regard to the further rejections presented by the Examiner, heparin may be a result effective variable. However, those skilled in the art will understand that this only holds true when concerning the intended use of the media as taught in Xia. A basic difference between the media of Xia and the present invention is in the intended use of the culture media. Depending on the use of the media, a variety of different components may be a result effective variable. The intended use is not necessarily the common technical feature among the groups. In addition, it is respectfully

submitted that in the field of art in which the present invention belongs, those skilled in the art will understand the importance of concentration levels of the different components of the culture media. That is, despite one culture media having common components as another culture media, it is wholly possible that the concentrations may result in opposing results (*e.g.*, promoting expansion versus preventing expansion). Thus, merely being a form "suitable" may not be enough to realize a different intended use.

III. THE 35 U.S.C. § 112 REJECTION SHOULD BE WITHDRAWN

The Examiner has rejected claims 31-33 and 36-40 under 35 U.S.C. § 112, second paragraph, for being indefinite. (See 1/19/10 Office Action, p. 3).

The Examiner states that since "the prior art measures protamine in concentration units of mg/ml which is contrary to Applicant's disclosure of UI/ml the metes and bounds of the claim are unclear as one of ordinary skill in the art measures these compounds differently." (See 6/23/10 Office Action, p. 3). It is respectfully maintained that the concentration units disclosed in the prior art is not the only standard in which to provide concentration units. The unit UI represents "International Unit" which those skilled in any scientific art would understand. Furthermore, anyone skilled in the art would understand that concentration units, or any similar units, may be converted into other forms of units. It is also unclear why the Examiner would state that a concentration unit of mg/ml is *contrary* to a concentration unit of UI/ml. The term concentration itself merely refers to an amount per unit volume which both units indicate. Regarding the specific case of UI (which is also referred to as IU in American standards), each compound has a respective conversion value determined by the World Health Organization. Likewise, protamine also holds a conversion from UI to any other unit measurement. Thus, claims 31 and 39 recite the UI/ml standard as those skilled in the art would fully understand and be clear as to the relationship between UI and mg relating to protamine.

Furthermore, the Examiner states that a conversion between mg and UI would be found persuasive in overcoming this rejection. It is respectfully submitted that the conversion between mg and UI for protamine is known in the art to be 1mg of protamine for 100 UI of protamine. Specifically, in item 4.2, first paragraph lines 3-5, "1 mg of protamine sulfate (1 ml) per 100 IU

of neutralize heparin is administered, if the time elapsed since the administration of heparin is less than 15 minutes." (See Hospira Productos Farmaceuticos Y Hospitalarios, S.L., Francisca Delgado, 11, 2ª planta, June 2007). As those skilled in the art understand the amount of protamine to neutralize heparin, the conversion results in the above ratio.

Accordingly, it is respectfully submitted that the range of "0.1 and 10,000 UI/ml protamine" is sufficiently described in the Specification at the time the application was filed as well as being sufficiently clear to one skilled in the art. Therefore, it is respectfully submitted that the Examiner should withdraw the 35 U.S.C. § 112 rejection for claims 31 and all dependent claims 32-33, 36-40.

IV. THE 35 U.S.C. §§ 102(b), 103(a) REJECTIONS SHOULD BE WITHDRAWN

The Examiner rejects claims 31-33 and 36-37 under 35 U.S.C. §§ 102(b), 103(a) as unpatentable over The Journal of Immunology, Xia et al., 2002 (hereinafter "Xia") in view of U.S. Pat. No. 4,735,726 to Duggins. (See 1/19/10 Office Action, p. 4).

To expedite processing of the present application, claim 31 has been amended to recite an "autologous culture medium for a culture in vitro, purification, and expansion of autologous human progenitor stem cells" "*consisting*" "between 0.1% and 90% weight of autologous human serum supplemented with between 0.1 and 10,000 UI/ml heparin and between 0.1 and 10,000 UI/ml protamine," and "a base culture medium including basic nutrients," "wherein the autologous human serum is obtained by plasmapheresis with heparin and protamine."

It is respectfully submitted that Xia and Duggins neither disclose or suggest an autologous culture medium that *consists* the autologous human serum in the recited concentrations and the base culture wherein the autologous human serum is obtained by plasmapheresis with heparin and protamine where the use is for a culture in vitro, purification, and expansion of autologous human progenitor stem cells, as recited in claim 31.

With reference to the previously presented arguments, it is respectfully maintained that the structure of the autologous serum of the present invention is different from that of Xia. The

structure (chemical composition) of the autologous serum obtained by plasmapheresis with heparin/protamine is unlike any obtained by other procedures, including plasmapheresis with Anticoagulant Citrate Dextrose Solution (ACD). One skilled in the art may obtain autologous serum manually, for example, by drawing blood from the patient with a syringe or mechanically by plasmapheresis. The most common way of obtaining autologous serum is the manual drawing of blood from the patient. While the serum isolating procedure is not specified in Xia, those skilled in the art may assume that that the serum was obtained in this manner. Among the instructions for manually drawing blood from the patient and isolating the serum in the drawn blood is allowing the blood to coagulate followed by centrifugation and microfiltration. The coagulation of blood requires the release of coagulation factors from the patelets. These factors remain soluble in the filtered serum and are inevitably present in the culture media used in laboratory research, such as that of Xia. Those skilled in the art will understand that serum allowed to clot naturally stimulates cell proliferation more than serum from which cells have been removed physically, to which patelet released factors account for this effect. Accordingly, the structural difference between the serum used in Xia and the serum of the present invention is a technical characteristic of the serum used to complement the media that affects cell physiology and accounts for a difference in the outcome of the cell culture exposed to the serum. Thus, the culture medium including the recited compositions is not disclosed in Xia.

Also as was previously argued, in an alternate embodiment, manually drawn blood may be treated with an anticoagulant. Xia explains that in order to isolate monocytes from healthy donors, venous blood is drawn and anticoagulated with a 3.8% sodium citrate solution. Thus, in the event that any assumption regarding the use of anticoagulants for obtaining autologous serum were to be made, it should be that sodium *citrate* is used as the anticoagulant. Autologous serum obtained by drawing blood manually with the use of sodium citrate as the anticoagulant is also structurally different from the serum used in the expansion media of the present invention, as will be explained in further detail below.

In the plasmapheresis procedure, whole blood is continuously removed from the patient through a central venous catheter and enters the pheresis machine through an extracorporeal circuit. Within the machine, cells are immediately separated from plasma by filtration,

suspended in replacement fluid and promptly returned to the subject's body, while plasma is put to its desired use. Despite no time being allowed for clotting, using an anticoagulant is highly recommended in plasmapheresis to allow the flow of blood from the patient's body to the pheresis machine, where commonly used anticoagulants include heparin and ACD. Once the plasmapheresis procedure is finished, protamine is given to the patient in order to neutralize heparin and allow for the normal physiology of reconstructed blood. A neutralizing dose of protamine will be added to the acellular filtered plasma. Then, plasma proteins will be allowed to clot, due to the presence of calcium, and cleared plasma retrieved by centrifugation. It is noted that acellular plasma protein coagulation does not involve the releasing of coagulating factors. This structural difference allows an optimal physiological environment for the cells being expanded for autologous drafting purposes.

Those skilled in the art will understand the biochemical basis for the anticoagulating action of ACD being the chelating of calcium by citrate. Acellular clotting of proteins will not occur in the absence of calcium, therefore the plasma obtained by plasmaphareris with ACD is structurally different from the plasma obtained by plasmapheresis with heparin/protamine because it contains additional proteins or increased concentration of proteins that may affect the fate of cells being expanded for autologous drafting purposes. For example, such proteins may act as adyuvants in the immune response to transplant rejection. Moreover, calcium is a signaling molecule for cell attachment, cell growth, and differentiation. The centrally positioned signaling molecule Ras is very sensitive to calcium levels. This small GTPase operates as a binary molecular switch and regulates cell proliferation and differentiation. A cell in culture decodes a variety of $InsP_3$-dependent Ca^{2+} signals in time, amplitude, and space during the process of cardiac cell differentiation and heart development. Studies performed in embryonic stem cell differentiating in cardiomyocytes have uncovered that Ca^{2+} regulates multiple steps of cell differentiation. These include secretion of cardiogenic factors, cardiac transcriptional cascades and in turn gene expression, myofibrillogenesis, and initiation of embryonic pacemaker activity. Thus, Ca^{2+} is a major second messenger directing the fate of stem cells. Its absence, due to the presence of chelating factors like citrate, undoubtedly affects the differentiation of progenitor stem cells and their gene expression which may lead to immune rejection upon transplantation.

Thus, it is respectfully submitted that the structure of the autologous serum specified in the expansion media as recited in claim 31 confers a special technical feature to the expansion media such that it results optimally for the use of expanding progenitor-stem cells for autologous drafting purposes, in particular with regards to the structure of the autologous serum. Accordingly, at the time the present application was filed, the media of the present application constitutes an alternative media suitable for expanding progenitor stem cells, in particular muscle progenitor stem cells.

It is again respectfully noted that it appears that Duggins is only cited to state that plasmapheresis is commonly used to obtain serum proteins and to produce cell culture media. However, Duggins does not disclose or suggest the culture media as recited in claim 31.

For at least the above described reasons, it is respectfully submitted that neither Xia nor Duggins, either alone or in combination, does not disclose or suggest the recitation of claim 31. Accordingly, it is respectfully submitted that claim 31 is allowable and the Examiner should withdraw the 35 U.S.C. §§ 102(b), 103(a) rejection for this claim. Because claims 32, 33, 36, and 37 depend from and, therefore, include the limitations of claim 31, it is respectfully submitted that these claims are also allowable.

V. THE 35 U.S.C. § 103(a) REJECTIONS SHOULD BE WITHDRAWN

The Examiner rejects claims 31-33 and 36-40 under 35 U.S.C. § 103(a) as unpatentable over U.S. Pat. Pub. No. 2002/0124855 to Chachques in view of U.S. Pat. No. 7,015,037 to Furcht et al. (hereinafter "Furcht") in further view of U.S. Pat. No. 4,735,726 to Duggins in further view of U.S. Pat. No. 6,624,141 to Yang et al. (hereinafter "Yang"). (See 6/23/10 Office Action, p. 9).

The Examiner maintains the previous rejection in view of the above references. Specifically, the Examiner states that the culture medium of Chachques emphasizes the importance of avoiding an immune response by using autologous cells and Furcht teaches that cardiac cells can be cultured with autologous serum. The previously presented arguments are

again being maintained. Furcht explicitly addresses the solutions for preventing immune rejection by disclosing specific approaches for transplantation to prevent immune rejection. (See Furcht, col. 28, l. 61 – col. 29, l. 25). In this discussion, Furcht *does not include* the use of autologous serum. Due to the detail involved in the description of this section, one skilled in the art would reasonably assume that autologous serum would have no effect on the outcome of cells expanding for drafting purposes in view of the teachings of Furcht. Thus, the likelihood of adding autologous serum to the media is not a reasonable combination between Chacques and Furcht. Furthermore, despite using plasmapheresis with heparin/protamine, the medium of the present application is not necessarily obvious. Despite one in the art potentially reaching the solution in order to provide for an alternative culture media that diminishes rejection upon transplantation (which is not conceded), the skilled person in the art would not have decided to combine the teachings in the cited documents as a solution to the problem of avoiding rejection.

Therefore, the cited references do not disclose or suggest a use of the culture medium as recited in claim 31. Thus, for the reasons discussed above, it is respectfully submitted that the cited references do not obviate the recitation of claim 31. Accordingly, it is respectfully submitted that claim 31 is allowable and the Examiner should withdraw the 35 U.S.C. § 103(a) rejection for this claim. Because claims 32, 33, and 36-40 depend from and, therefore, include the limitations of claim 31, it is respectfully submitted that these claims are also allowable.

The Examiner rejects claims 31-33 and 36-40 under 35 U.S.C. § 103(a) as unpatentable over U.S. Pat. No. 6,472,212 to Valerio et al. (hereinafter "Valerio") in view of U.S. Pat. No. 5,817,773 to Wilson et al. (hereinafter "Wilson") in further view of Duggins. (See 6/23/10 Office Action, p. 12).

It was previously argued and is presently being maintained that those skilled in the art will understand that in the field that bone marrow cells and, in general, cells of the blood lineage, do not need attachment to substrates in order to be expanded. That is, these cells grow in *suspension*. This is an important property or technical character that is taken into account when designing media composition because culture media designed for suspension-growing cells may not support attachment dependent growing cells. The Examiner responds by stating that

modifying a timing of the addition of supplements is a matter of routine optimization and experimentation, thereby rendering a timing issue obvious. However, as discussed above, the issuc of intended use is revisited. Because the field of bone marrow cells or cells of the blood lineage do *not* need attachment to substrates in order to be expanded, one skilled in the art would *not* reasonably be led to the purportedly obvious conclusion that the Examiner states otherwise. Those skilled in the art will understand that progenitor stem cells are attachment-dependent. This limitation is a technical characteristic of the present invention. Culture media that does not allow attachment of cells or permits only poor attachment of cultured cells to the plate surface will result in a high rate of detachment and death of expanding progenitor stem cells, thereby precluding expansion to cell counts suitable for transplantation. The claimed media composition presents high autologous calcium content which is critical for cell attachment. The presence of autologous calcium in the media is a consequence of the novel combination of media components as that recited in claim 31. Specifically, as described above, the autologous serum chosen by its isolation method (*i.e.*, plasmapheresis with heparin/protamine) results in high levels of autologous calcium in the final media composition.

It was previously noted that it appears that the Examiner is constructing the claimed media by adding unconnected references solely on the basis that they contain the element that is suitable to obtain the claimed media regardless of the functionality that component may provide the respective reference. Thus, it was respectfully submitted that the functionality of the component is a critical factor that is part of the culture media and that the Examiner's attempt at randomly selecting references to obviate claim 31 is impermissible hindsight.

The Examiner also states that the argument regarding the order in which the components of the media being critical is merely argument of counsel and unsupported. (See 1/19/10 Office Action, p. 19). This argument is respectfully being maintained and is not merely argument of counsel as it is based on objective evidence. As discussed previously, it is not reasonable to add the histamine to the protamine-containing medium of Valerio because the medium was designed to have a particular physiological effect on the growing cells. This physiological effect is a technical characteristic of the media and it is provided by its protamine content. Those skilled in the art would understand that a selection of a starting media with technical characteristics that are

suitable for solving the problem is critical in attempting to find a final media. To subsequently neutralize the technical characteristic that was specifically selected for the trials would run contrary to basic experimentation principles. In the interest of objectivity (thus the present Specification is not noted), these principles are founded at least in the prior art references listed in ¶ [0006] as well as in Xia which discusses lengths of time in which components are added and further components being added thereafter (See Xia, p. 1132).

Furthermore, it is respectfully submitted that Valerio discloses that the culture medium described therein is supplemented with IL3. (See Valerio, col. 29). Those skilled in the art will understand that IL3 induces cell differentiation so that stem cells *cannot* be maintained, especially with myoblasts. Therefore, the use of the culture medium of Valerio in combination with any other teachings would not lead one of ordinary skill in the art to obviate the recitation of claim 31.

Therefore, the cited references do not disclose or suggest a use of the culture medium as recited in claim 31. Thus, for at least the above reasons, it is respectfully submitted that claim 31 is allowable and the Examiner should withdraw the 35 U.S.C. § 103(a) rejection for this claim. Because claims 32, 33, and 36-40 depend from and, therefore, include the limitations of claim 31, it is respectfully submitted that these claims are also allowable.

CONCLUSION

In light of the foregoing, Applicants respectfully submit that all of the now pending claims are in condition for allowance. All issues raised by the Examiner having been addressed, and an early and favorable action on the merits is earnestly solicited.

Respectfully submitted,

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